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Review

Monocyte chemoattractant proteins in the pathogenesis of systemic sclerosis

J. H. W. Distler¹, A. Akhmetshina¹, G. Schett¹ and O. Distler²

Activation of the immune system and increased synthesis of extracellular matrix proteins by fibroblasts are hallmarks in the pathogenesis of SSc. The molecular mechanisms underlying the infiltration of inflammatory cells into the skin and the subsequent activation of fibroblasts are still largely unknown. Chemokines are a family of small molecules that are classified according to the position of the NH₂-terminal cysteine motif. Recent data indicate that chemokines and in particular two members of the subfamily of monocyte chemoattractant proteins, MCP-1 (CCL-2) and MCP-3 (CCL-7), might be involved in the pathogenesis of SSc. MCP-1 and -3 are overexpressed by SSc fibroblasts and in skin lesions from SSc patients compared to healthy controls. MCP-1 and -3 are chemotactic for inflammatory cells and stimulate their migration into the skin. In addition to their pro-inflammatory effects, MCP-1 and -3 contribute to tissue fibrosis by activating the synthesis of extracellular matrix proteins in SSc fibroblasts. Therapeutic strategies targeting MCP-1 have revealed promising results in several animal models of SSc. Antagonists against the receptor CCR2 are currently tested in clinical trials of a variety of diseases and also represent interesting candidates for target-directed therapy in SSc.

KEY WORDS: Chemokines, Chemokine receptors, Systemic sclerosis, MCP-1.

Introduction

SSc is a generalized fibrotic disease of unknown aetiology, which affects the skin and various internal organs. Histopathological hallmarks of SSc are inflammatory infiltrates in early disease stages and an accumulation of extracellular matrix proteins leading to tissue fibrosis. Inflammatory infiltrates are dominated by macrophages and T cells, and are localized preferentially in perivascular areas. Inflammatory cells migrate into the skin before a significant accumulation of extracellular matrix can be observed. Tissue fibrosis in SSc is caused by an overproduction of extracellular matrix components by activated fibroblasts [1]. SSc fibroblasts produce increased amounts of extracellular matrix proteins that accumulate in the skin [2]. Although significant progress has been made in analysing the different components of the extracellular matrix, the mechanisms for the pathological activation of fibroblasts in SSc have only partially been identified. Most authors favour an interaction of different cell types orchestrated by pro-inflammatory and pro-fibrotic cytokines. This concept is supported by the observation that fibroblasts, which produce the highest amount of extracellular matrix proteins, are often localized in close proximity to the inflammatory infiltrates [1]. According to this hypothesis, pro-inflammatory cytokines such as MCP-1 are released in early disease stages of SSc. The pro-inflammatory cytokines attract T cells and mononuclear cells to the skin, which in turn release pro-fibrotic cytokines such as TGF- β and IL-4 [1]. These pro-fibrotic cytokines then activate in turn the synthesis of extracellular matrix in dermal fibroblasts leading to tissue fibrosis in later stages of the disease. However, the mechanisms that stimulate the initial release of pro-inflammatory cytokines, which then trigger the migration and activation of inflammatory cells in the early pathogenesis of SSc, remain elusive.

Structure and classification of chemokines

Chemokines are a family of small molecules with a molecular weight of 8–14 kDa. To date, ~50 human chemokines and 20 G-protein-coupled chemokine receptors have been identified (see supplementary tables 1–3, available as supplementary data at *Rheumatology* Online). Based on their genetic organization and the position of two highly conserved cysteine residues at the N-terminus, chemokines can be divided into four subgroups, the CC, CXC, C and CX₃C families [3]. In the CXC chemokine family, the two N-terminal cysteine residues are separated by a variable amino acid. Genes from this family are clustered at human chromosome 14q12–21. Based on the presence or absence of a glutamic acid–leucine–arginine motif at the N-terminus, CXC chemokines can be subclassified into ELR+ and ELR– molecules. Chemokines of the CC family have adjacent cysteines close to the N-terminus, and their genes cluster—with some exceptions—at 17q11.2–12 [4]. As a general rule, members of the CC family are primarily targeting monocytes and T cells, whereas CXC chemokines affect mainly neutrophils. The two members of the C chemokine family, lymphotactin- α and lymphotactin- β , are characterized by a single cysteine residue at the N-terminus. The only member of the CX₃C chemokine family, fractalkine, has a typical chemokine domain at its N-terminus except for the presence of three amino acids between the first two cysteines. Fractalkine is the only chemokine that is membrane-bound [5]. Besides the chemokine domain, fractalkine also consists of a mucin-like stalk, a transmembrane domain, which anchors it to the plasma membrane, and a cytoplasmic domain. An alternative classification system distinguishes inducible chemokines with pro-inflammatory properties from homeostatic cytokines, which are constitutively expressed [6].

Chemokine receptors

Chemokine receptors are seven-transmembrane-domain receptors, which are coupled to heterotrimeric G-proteins [7]. To induce migration of target cells along a chemotactic gradient, the release of the $\beta\gamma$ subunits from the heterotrimeric G-protein is required. Among other pathways, the $\beta\gamma$ subunits then directly activate phosphoinositide-specific phospholipase C (PLC) isoenzymes leading to the formation of inositol-1,4,5-trisphosphate and

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a rise of the intracellular calcium concentration. The signals mediated by chemokine receptors (except CXCR 4) are short and transient. The rapid termination of receptor activity is achieved by phosphorylation at multiple sites of the cytoplasmic C-terminus, homologous and heterologous desensitization and subsequent internalization [7]. It is noteworthy that the chemokine/chemokine receptor system is highly redundant, in that most chemokine receptors bind several different chemokines and most chemokines bind to several different receptors (see supplementary tables 1–3, available as supplementary data at *Rheumatology* Online).

Function of chemokines

During inflammation, sentinel cells at the inflammatory focus release chemokines and generate a chemotactic gradient to surrounding blood vessels. Presentation of chemokines on endothelial cells induces rolling and transendothelial migration of leucocytes. For the migration along the chemotactic gradient, sensing of subtle differences in the concentration of chemokines is required. This occurs via the accumulation of intracellular signalling molecules at the leading edge of migrating cells, whereas the chemokine receptors themselves remain uniformly distributed over the plasma membrane during migration [3, 8]. The mechanism whereby the chemotactic gradient leads to the asymmetric distribution of intracellular signalling molecules remains to be elucidated.

Although leucocyte chemotaxis and lymphocyte development are the most prominent features of chemokines, chemokines also possess other biological activities [3]. Of particular interest for SSc, chemokines are also involved in the regulation of angiogenesis and vasculogenesis. A structural domain of the CXC chemokine family determines their angiogenic potential [9, 10]. The N-terminus of CXC family members such as IL-8 (CXCL8) and growth-related oncogenes (GROs) α , β and γ (CXCL1–3), contain three amino acid residues (Glu–Leu–Arg), the so-called ELR motif. These ELR+ CXC chemokines have strong chemotactic effects on endothelial cells and can induce neovascularization *in vivo*, even in the absence of leucocytes. In contrast, ELR– CXC chemokines, such as platelet factor 4 (CXCL4) and monokine induced by IFN- γ (MIG, CXCL9), are potent inhibitors of angiogenesis. Other functions of chemokines include control of cell proliferation, alteration of the expression of adhesion molecules and regulation of interactions between HIV and target cells [10].

Expression of MCP-1 in the skin of SSc patients

Five members of the family of monocyte chemoattractant proteins have been identified so far. MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13) and MCP-5 (CCL12) constitute a subfamily within the CC chemokines [11, 12]. The amino acid sequences among the monocyte chemoattractant proteins are highly conserved with up to 70% homology. They belong to the functional class of inducible, pro-inflammatory chemokines and have been shown to be chemotactic for monocytes, eosinophils and basophils [11]. Two members of the family of monocyte chemoattractant proteins, MCP-1 and -3, have been linked to the pathogenesis of SSc.

MCP-1 (CCL2) is a 99-amino acid protein that has been shown to be identical to the murine JE gene product [13]. MCP-1 can be released by a large variety of cells including fibroblasts, epithelial cells, mononuclear cells, endothelial cells and smooth muscle cells [14–16]. In SSc, numerous groups have shown that MCP-1 is expressed abundantly in the skin of patients on the mRNA and protein level [17–20]. In skin biopsies from involved skin areas, MCP-1 expression was detected by *in situ* hybridization and immunohistochemistry in mononuclear cells, fibroblasts, endothelial cells and keratinocytes. Interestingly, a similar expression pattern for MCP-1 was also found at non-fibrotic skin sites,

suggesting that MCP-1 might play a role in the initiation of tissue fibrosis [17]. In early, inflammatory stages, MCP-1 was expressed mainly by infiltrating mononuclear cells, whereas in later, fibrotic disease stages, fibroblasts became the major source for MCP-1 in the dermis of SSc patients. In contrast, the expression of MCP-1 mRNA in the skin of healthy individuals was limited to a few scattered epidermal and dermal cells and no expression of MCP-1 protein was detectable on the protein levels by immunohistochemistry.

In contrast to the situation *in vivo*, a constitutive expression of MCP-1 mRNA and protein was detected in cultured fibroblasts not only from SSc fibroblasts, but also from healthy controls [17]. The expression of MCP-1 was not only found in the presence of fetal calf serum, but also after growth factor deprivation. The levels of platelet-derived growth factor (PDGF) are elevated in SSc patients and are thought to contribute to the pathogenesis of SSc. We could demonstrate that PDGF induces dose-dependently the expression of MCP-1 in cultured dermal fibroblasts. Concentrations up to 10 ng/ml PDGF significantly induced MCP-1 mRNA compared with unstimulated fibroblast cultures. In contrast to the data obtained for MCP-1 protein, no further increase of MCP-1 mRNA was observed with higher concentrations of PDGF. These data indicate that at higher concentrations of PDGF, the induction of MCP-1 might be driven by increased translation, in addition to increased transcription [17].

Karrer *et al.* [21] analysed in a limited number of German SSc patients the frequency of the –2518 G single nucleotide polymorphism of the MCP-1 gene. They found that the GG genotype was more frequent in their collection of SSc patients than in healthy volunteers. Of particular interest, the expression of MCP-1 was higher in GG homozygous patients than in patients with the AG or the AA genotype. However, no significant differences in the prevalence of this polymorphism were found in two larger follow-up studies from the United Kingdom and from the Czech Republic [22, 23].

MCP-1 in fibrotic diseases

In addition to increased expression in the skin, MCP-1 levels are also increased in the blood of SSc patients [19, 24–27]. Serum levels of MCP-1 might be particularly high in patients with early and diffuse disease [25, 26]. However, another report did not find changes in the serum concentrations of MCP-1 over time [24]. High serum levels of MCP-1 might also identify SSc patients with increased risk of major organ involvement. Three groups reported independently that serum levels of MCP-1 were higher in patients with pulmonary fibrosis [19, 25, 27]. In addition, correlations with renal and cardiac involvement have been described [25, 27].

MCP-1 is expressed in SSc from very early stages on and it is a potent chemoattractant for mononuclear cells [28]. Local expression of MCP-1 in the skin of SSc patients might establish a chemotactic gradient guiding inflammatory cells into the skin of SSc patients. Indeed, experiments with blocking antibodies against MCP-1 revealed that cultured fibroblasts from SSc patients promote migration of mononuclear leucocytes by the release of biologically active MCP-1 [17, 29]. MCP-1 might therefore play a key role in the infiltration of the skin by mononuclear cells and the development of inflammatory infiltrates in early stages of SSc, which might stimulate in turn the activation of resident fibroblasts.

Indeed, inhibition of MCP-1 exerts potent anti-fibrotic effects in several inflammation-driven models of tissue fibrosis. In a murine model of crescentic nephritis, a strong induction of MCP-1 was observed that was accompanied by an influx of inflammatory cells and the development of fibrosis [30]. Blocking MCP-1 by anti-MCP-1 antibodies resulted in a reduced tissue infiltration by inflammatory cells and a decreased deposition of type I collagen in the kidney [31]. MCP-1 has also been suggested to play a role in experimental pulmonary fibrosis. In bleomycin-induced lung fibrosis, MCP-1 is expressed in a time-dependent manner after

exposure to bleomycin with highest levels of MCP-1 observed in areas of active fibrosis [32]. MCP-1 also plays a key role in the pathogenesis of bleomycin-induced dermal fibrosis, a common model of SSc. Mice lacking MCP-1 are protected from bleomycin-induced dermal fibrosis [33]. The number of inflammatory cells and the accumulation of collagen in lesional skin of mice lacking MCP-1 upon bleomycin challenge were strongly reduced compared with wild-type mice. Furthermore, treatment with SKL-2841, a small molecule antagonist of MCP-1 and MIP-1 β , prevented bleomycin-induced dermal fibrosis [34].

The important role of MCP-1 in pulmonary fibrosis was supported by studies on mice lacking CCR2, the receptor for MCP-1. After intratracheal instillation of bleomycin or FITC, the hydroxyproline content of the lungs was significantly reduced in CCR2 knockout animals compared with wild-type mice and CCR2 knockout mice were protected from fibrosis [35, 36].

Activation of CCR2 signalling by MCP-1 and -5 might also play an important role for the migration of fibrocytes into affected tissues in fibrotic diseases. Fibrocytes are bone marrow derived, circulating fibroblast-like cells that can be identified by their expression of the haematopoietic stem cell antigen CD34, the common leucocyte antigen CD45 and type I collagen. In culture, these cells can acquire a myofibroblast phenotype and produce large amounts of collagen. Fibrocytes have been implicated in the pathogenesis of fibrotic disease in particular in pulmonary fibrosis [37]. Murine fibrocytes isolated from fibrotic lungs expressed CCR2 and migrated towards the CCR2 ligands MCP-1 and -5 *in vitro* [38]. In mice lacking CCR2, fibrocyte recruitment was reduced upon challenge with intrapulmonary FITC. Recruitment of fibrocytes in CCR2 knockout mice was restored, when CCR2 knockout mice were transplanted with bone marrow from wild-type mice [38]. These findings demonstrate an important role for CCR2 for the recruitment of fibrocytes in mice. However, CCR2 signalling might be less important in humans. First, human fibrocytes express lower levels of CCR2 than murine fibrocytes [39]. Second, no human homologue of the CCR2 agonist MCP-5 has been identified so far and MCP-5 might only be relevant to murine biology.

Direct effects of MCP-1 on fibroblasts

Whether MCP-1 is directly involved in the activation of dermal fibroblasts leading to the accumulation of extracellular matrix proteins in SSc is controversial. We did not observe any effects of recombinant MCP-1 on the synthesis of type I collagen in our fibroblast cultures as assessed by real-time PCR, radioimmunoassay for the N-terminal propeptide of type I collagen and direct measurements of the collagen concentrations with the SirCol collagen assay [17, 40]. In addition, blocking antibodies to MCP-1 did not reduce the basal collagen synthesis of cultured fibroblasts from SSc patients and healthy controls [40]. Our observations were confirmed by Galindo and coworkers [18], who also did not observe stimulatory effects of MCP-1 on cultured dermal fibroblasts. Consistently, the receptor for MCP-1, CCR2, was not detectable in our fibroblasts by real-time PCR, *in situ* hybridization and FACS [40]. In contrast, Yamamoto *et al.* [41] detected a significant increase of $\alpha 1(I)$ collagen mRNA after incubation of cultured dermal fibroblasts with recombinant MCP-1 by northern blot hybridization. A possible explanation for these distinct findings might be different cell sources, since Yamamoto *et al.* used outgrowth cultures, whereas fibroblasts were obtained from biopsies by enzymatic digestion in the other studies. Alternatively, fibroblasts might express CCR2 only during a short time-frame in the course of the disease and then lose the expression of receptors for MCP-1. Along this line, Carulli *et al.* [42] detected signals for CCR2 by flow cytometry only in a subset of fibroblast cultures established from patients with early SSc. Consistently, expression of CCR2 was detected only in patients with early, diffuse disease by immunohistochemistry. Double labelling experiments demonstrated that

CCR2-positive fibroblasts expressed α -smooth muscle actin, suggesting that the expression of CCR2 might be restricted to myofibroblasts [42]. Of interest, CCR2 positive fibroblasts also overexpressed MCP-1, suggesting a possible autocrine activation loop in myofibroblasts. Indeed, inhibition of MCP-1 or CCR2 attenuated the expression of α -smooth muscle actin. Thus, MCP-1 might stimulate the differentiation of a subset of resting fibroblasts into metabolically active myofibroblasts in an autocrine manner in early stages of SSc.

In addition to potential effects on collagen production, MCP-1 might also alter matrix turnover by regulating the expression of matrix-degrading enzymes and their inhibitors. Yamamoto and coworkers [43] demonstrated that MCP-1 up-regulates the expression of tissue inhibitor of MMP-1 (TIMP-1) in dermal fibroblasts on the mRNA and on the protein level. Of note, the expression of MMP-1 and -2 was also increased. The induction of MMP-1 might partially be mediated by IL-1. MCP-1 stimulated the expression of IL-1 in parallel to MMP-1 and pre-incubation with IL-1 receptor antagonist reduced the stimulatory effects of MCP-1 on MMP-1 [43].

Alternative receptors for MCP-1 in SSc

Since only a subset of fibroblasts might express CCR2 for a short time period, the expression of alternative receptors for MCP-1 in SSc patients was analysed. Besides CCR2, three other receptors have been shown to bind MCP-1, namely D6, the Duffy antigen receptor for chemokines (DARC) and US28. In contrast to CCR2, all of these receptors are not specific for MCP-1, but bind several other cytokines with similar affinity. D6 is a seven-transmembrane-spanning receptor that is most closely related to CCR4 and IL-8R [44]. DARC was first identified as the Duffy antigen on red blood cells. However, DARC is not only expressed by erythrocytes, but also by numerous other cell types including fibroblasts. Furthermore, the expression of DARC is not limited to Duffy antigen-positive individuals, but also DARC can be found in individuals whose erythrocytes do not express the Duffy antigen [45]. To date, no signalling has been observed upon binding of ligands to these receptors and D6 and DARC have been suggested to function as decoy receptors [45, 46]. Consistent with this hypothesis, mice lacking D6 exhibit severe hyperinflammatory responses. In contrast to D6 and DARC, US28 protein is a functional chemokine receptor [47, 48]. US28 protein is encoded by the open reading frame 28 of the CMV [47, 48] and has been shown to accelerate inflammation in the presence of MCP-1 [49]. D6 and US28 were not expressed in the skin of SSc patients or controls. mRNA for DARC was detectable in SSc fibroblasts *in vitro* and *in vivo*. However, the expression levels were low and no differences were observed between SSc patients and healthy individuals [40]. Together with the fact that no signalling has been detected upon chemokine binding to DARC, these data suggest that DARC does not contribute to the activation of profibrotic signalling cascades by MCP-1 in SSc fibroblasts. In summary, we did not detect any functional receptor for MCP-1 on fibroblasts and direct stimulation of fibroblasts with MCP-1 did not induce the synthesis of collagen.

Indirect, T-cell-dependent induction of collagen by MCP-1

MCP-1 has consistently been shown to be overexpressed in SSc. Furthermore, animal models of different fibrotic diseases implicate a pro-fibrotic role of MCP-1, but direct stimulatory effects of MCP-1 on the collagen production by SSc fibroblasts have been observed only in a subset of fibroblast cultures. Thus, the profibrotic effects of MCP-1 may require interactions with other cell types such as T cells. In skin specimens of patients with SSc, Mavalia *et al.* [50] found a predominance of Th2 cells, and there is increasing evidence that Th2 cytokines contribute to the deposition of collagen in fibrotic diseases such as SSc. Among the Th2

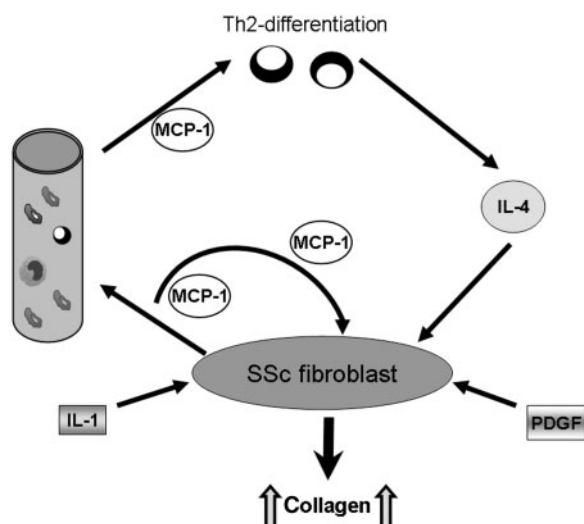


FIG. 1. Proposed function of MCP-1 (CCL2) in the pathogenesis of systemic sclerosis. Dermal fibroblasts act as sentinel cells and release MCP-1 in early stages of SSc. PDGF and IL-1 β contribute to the induction of MCP-1 in dermal fibroblasts, resulting in a further increase of the expression of MCP-1. MCP-1 attracts mononuclear cells across the endothelial cell barrier into the dermis. MCP-1 might stimulate directly the expression of collagen in a subset of SSc fibroblasts. MCP-1 contributes also indirectly to the accumulation of extracellular matrix in SSc. MCP-1 induces a Th2 differentiation in infiltrating Th0 cells. Th2 cells release increased amounts of IL-4, which stimulates in turn the collagen synthesis in SSc fibroblasts. In this model, glycosaminoglycans function as a local reservoir for MCP-1. (Figure modified from [40]).

cytokines, IL-4 is thought to play an important role in the pathogenesis of SSc. The levels of IL-4 are increased in the serum of SSc patients [51] and IL-4 is also overexpressed in the skin of SSc patients [52]. Furthermore, IL-4 has been shown to stimulate the synthesis of extracellular matrix proteins in dermal fibroblasts directly and potentially [40, 53]. The importance of IL-4 for the development of fibrosis *in vivo* is emphasized by the observation that treatment with neutralizing anti-IL-4 antibodies prevents the deposition of collagen in the tight-skin-1 mouse model of SSc and leads to normalization of the dermal collagen content [54].

Consistent with the theory of an indirect role of MCP-1 in fibrotic processes, we recently demonstrated that MCP-1 can induce a Th2 differentiation in Th0 cells resulting in an increased release of IL-4 (Fig. 1) [40]. When Th0 cells were stimulated for 7 days by IL-2 and anti-CD3/CD28, the level of IL-4 released by these T cells was significantly higher in the presence of MCP-1. The level of MCP-1 increased further when the cells were re-stimulated with MCP-1 and anti-CD3/CD28 after a resting period of 4 days. We could also show that glycosaminoglycans function as a local reservoir for MCP-1 in the skin of SSc patients [40]. Like other components of the extracellular matrix, glycosaminoglycans are greatly enriched in the skin of patients with SSc compared with healthy controls. The positively charged C-terminus of MCP-1 can bind to negatively charged glycosaminoglycans via ionic interactions [55, 56]. We could demonstrate that MCP-1, which is re-released from glycosaminoglycans in a time-dependent manner, is capable of stimulating efficiently the production of IL-4 in Th0 cells [40].

Thus, direct and indirect effects might contribute to the pro-fibrotic effects of MCP-1 in SSc. MCP-1 might directly stimulate the collagen production in a subset of patients in early stages of the disease. In other patients or in other stages of the disease, the pro-fibrotic effects of MCP-1 might preferentially indirectly be stimulating the release of IL-4 from T cells.

MCP-3 in the pathogenesis of SSc

MCP-3 (CCL7) is an 11 kDa protein that is closely related to MCP-1. The chemotactic potency of MCP-3 for monocytic cells is

similar to that of MCP-1. However, the chemotactic effects of MCP-3 are broader than that of MCP-1. Since MCP-3 binds not only to CCR2, but also with high affinities to CCR1 and CCR3 [11], MCP-3 is also chemotactic for dendritic cells and granulocytes in addition to monocytes, NK cells and lymphocytes [11, 57]. The expression patterns of MCP-3 are similar to MCP-1, but MCP-3 is released in lower levels by dermal fibroblasts than MCP-1 [58].

Tight-skin mice-1 (Tsk-1) are widely used as animal models of SSc. Ong and co-workers [59] recently demonstrated that MCP-3 mRNA and protein are highly up-regulated in cultured fibroblasts of neonatal Tsk. Immunohistochemistry demonstrated an abundant expression of MCP-3 in the dermis of Tsk-1 mice. In contrast, the expression of MCP-3 was limited to perifollicular cells in control mice. In SSc patients, MCP-3 was expressed by mononuclear cells, dermal fibroblasts and basal keratinocytes of all patients. In healthy individuals, MCP-3 was detected only in basal keratinocytes. The release of MCP-3 was stimulated by TGF- β in murine fibroblasts [59]. The induction of MCP-3 by TGF- β depended partially on p42/44 ERK activation, since inhibitors of p42/44 ERK prevented the up-regulation. MCP-3 might also play a role in the pathological activation of skin fibroblasts in SSc. In reporter assays, the promoter of the murine pro α 2(I) collagen gene is activated by MCP-3 [59]. It has not been determined yet, whether MCP-3 can up-regulate the collagen synthesis indirectly by inducing the release of pro-fibrotic mediators by T cells. It has not been determined yet, whether MCP-3 might also stimulate Th2 polarization of T cells. Although the importance of MCP-3 needs to be confirmed in animal models for tissue fibrosis, MCP-3 might play an important role in the accumulation of extracellular matrix in SSc.

Implications for therapy

During the last years, blocking of chemokine signalling has gained growing interest as a potential new therapeutic strategy. In general, the action of chemokines can be blocked by chemokine-specific neutralizing antibodies, chemokine receptor antagonists or the inhibition of intracellular signalling pathways, which are activated by binding of chemokines to their receptors [60]. The proof of concept that antagonizing of chemokines is a valid therapeutic approach has been shown in animal models for a variety of diseases. For example, administration of an N-terminal-truncated MCP-1 analogue that exerts antagonistic effects on CCR2 prevents the onset of arthritis in the MRL-*lpr* mouse model of RA [61]. Furthermore, BX 471, a potent and specific CCR1 antagonist, has been developed, which can be administered orally [62]. A new class of small molecule CCR2 antagonists was recently evaluated [63, 64]. These small molecule CCR2 antagonists are potent inhibitors of monocyte responses to MCP-1, and they are specific for CCR2 and have appropriate pharmacokinetics for oral applications *in vivo*. The efficiency of these antagonists was investigated in several inflammatory conditions *in vivo*. In a mouse model for delayed type hypersensitivity, the rodent-active CCR2 antagonist INCB3344 suppressed the influx of macrophages and other inflammatory cells. Similar results were obtained with the related inhibitor INCB3268 in primates [64]. These inhibitors also blocked dose-dependently the influx of macrophages into the peritoneal cavity after thioglycolate challenge [63]. Furthermore, treatment with INCB3344, has been shown to reduce the clinical signs of adjuvant arthritis efficiently, when initiated at the onset of arthritis. Histological analysis demonstrated not only a marked suppression of inflammation, but also a significant reduction of bone resorption [63]. The authors claimed that CCR2 antagonists might selectively inhibit the inflammatory phases, but not affecting immune activation, and that CCR2 inhibitors might therefore represent a novel class of anti-inflammatory drugs without severe immunosuppressive side-effects. Because of the

TABLE 1. Companies with CCR2 antagonists and current status of clinical development

	Name of CCR2 antagonist	Type of trial	Reference
Incyte Corporation	INCB 8696	Phase I, planned for multiple sclerosis	http://www.incyte.com
Pfizer	PF-4136309	Phase II, pain in knee osteoarthritis	http://www.clinicaltrials.gov
ChemoCentryx, Inc.	CCX140	Phase I	http://www.chemocentryx.com
Bristol-Myers Squibb	BMS-741672	Proof of confidence in Insulin resistance	http://www.clinicaltrials.gov
		Phase II, for neuropathic pain	http://ctr.bms.com/
Millennium Pharmaceuticals	MLN1202	Phase II, of patients at high risk for atherosclerosis completed	http://www.mlnm.com
		RCT with RA completed	Vergunst <i>et al.</i> [66]

This list is not exhaustive. RCT: randomized controlled trial.

encouraging animal data, this group of small molecule CCR2 inhibitors is currently evaluated in clinical trials [63].

Harrington and colleagues [65] chose a different approach to target MCP-1 signalling. Instead of using a small molecule inhibitor of CCR2, they used monoclonal human anti-MCP-1 antibodies (ABN912) and evaluated their efficacy for the treatment of RA in a placebo-controlled dose escalation trial. ABN912 was well tolerated with no differences in the incidence of adverse events between placebo and treatment groups. However, after an initial drop, the total serum levels of MCP-1 unexpectedly increased dose-dependently up to 2000-fold in patients treated with ABN912 and remained elevated throughout the observation period of 180 days [65]. These effects might be explained by rapid binding of intravascular MCP-1 by ABN912 and subsequent mobilization of MCP-1 from extravascular compartments, e.g. from glycosaminoglycans. Consistent with the increase in circulating MCP-1, no clinical improvement was seen in patients treated with ABN912. Instead, the serum levels of CRP and the number of sublining macrophages tended to increase in patients treated with ABN912. The results of this study do question the strategy of targeting MCP-1 with neutralizing antibodies, because subsequent increases in serum levels might be a general phenomenon for MCP-1 and all other chemokines that bind to glycosaminoglycans, and are therefore stored in large quantities in the extracellular matrix. In general, strategies targeting CCR2 might be more potent than inhibition of MCP-1 for two reasons: (i) inhibitors of CCR2 should not lead to a mobilization of MCP-1 from extravascular stores, (ii) CCR2 is also a receptor for MCP-3 and inhibition of CCR2 thus targets simultaneously two pro-fibrotic cytokines, MCP-1 and -3. CCR2 antagonists are currently developed by a number of different pharmaceutical companies and are tested in clinical trials for various diseases. Table 1 summarizes the current status of clinical testing of these CCR2 antagonists. However, a recent trial with the human CCR2 blocking antibody MLN1202 in patients with RA failed to show improvement [66]. Treatment with MLN1202 reduced the levels of free CCR2 on synovial monocytes, but did not alter the expression of synovial biomarkers or the clinical course. This controlled Phase IIa trial was underpowered with only seven to nine patients per group and was thus not designed to detect clinical outcome. Furthermore, the observation time was limited to 6 weeks. Prolonged treatment might be needed before beneficial effects can be detected. Finally, the authors also focused on markers of monocyte activation and did not analyse fibroblasts and T cells, which are thought to mediate the pro-fibrotic effects of MCPs in SSc. Thus, the results of this trial do not exclude inefficacy of CCR2 antagonists in SSc.

Summary

MCP-1 and -3 have been characterized as important players in the pathogenesis of SSc that might control inflammation of fibrosis. MCP-1 is overexpressed in the skin of SSc patients and is able to initiate the migration of inflammatory cells from the blood into

involved tissues. MCP-1 plays also an important role in the development of skin fibrosis in SSc. MCP-1 induces a Th2 differentiation in Th0 cells. Th2 cells release increased amounts of IL-4, which stimulates in turn the collagen synthesis in dermal fibroblasts. In addition, MCP-1 might stimulate directly the production of extracellular matrix proteins in a subset of SSc fibroblasts. MCP-3 is overexpressed as well in SSc and activates collagen production in dermal fibroblasts. Targeting of MCP-1 and -3 might offer a great potential for novel therapeutic agents. Inhibitors of the MCP receptor CCR2 have been developed recently and are currently tested in clinical trials.

Rheumatology key messages

- MCP-1 and -3 are overexpressed by SSc fibroblasts and in skin lesions from SSc patients.
- Inhibition of MCP-1 signalling had promising anti-fibrotic effects in several animal models of SSc.
- Antagonists against the receptor CCR2 might represent interesting candidates for target-directed therapy in SSc.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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